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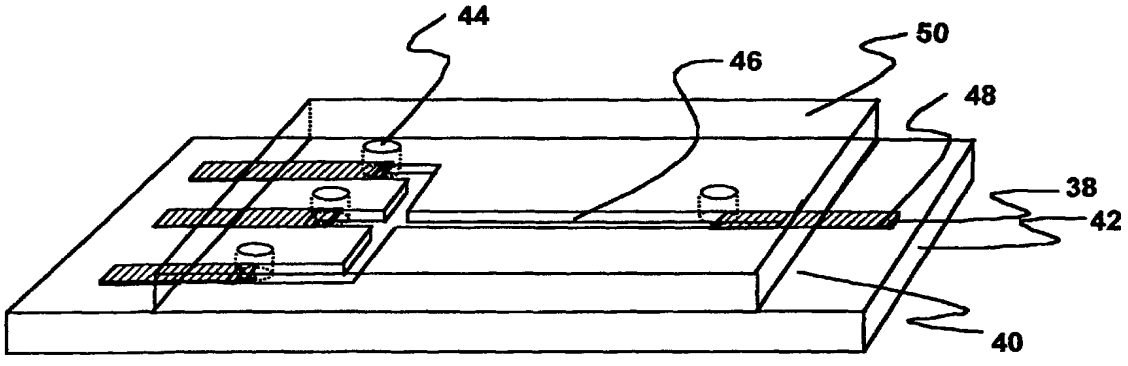

(57) Abstract: Microfluidic structures with at least one internal channel for fluid retention. In one embodiment, the microfluidic structure is a card-like chip comprising a substrate and a cover laminated thereon. The substrate is preferably a thin planar structure with at least one fluid channel formed on at least one side. A flexible chip is produced when a thin glass plate is used as the substrate. In another embodiment, the chip is an integral piece of plastic containing internal channels for fluid retention. This plastic chip may be provided with access holes for external access to the channels, and electrically conductive electrodes to allow electrical connection between fluid in the channel and the exterior. The plastic chip may be made of thermoplastic or thermoset resin.

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MICROFLUIDIC STRUCTURES AND METHODS OF FABRICATION

FIELD OF THE INVENTION

The present invention relates to microfluidic structures. In particular, the present invention relates to a geometric microstructure defining a liquid flow system.

BACKGROUND OF THE INVENTION

The current direction in analytical science is gearing towards miniaturisation of the total analysis system. This is also very often described as lab-on-chip; where a network of microfluidic structure, i.e. a geometric microstructure defining a liquid flow system, is found. Microfabricated lab-on-chips combine sample handling and analysis steps into a single package. Such an approach is commonly known as micro total analysis system (TAS). TAS is an ideal approach for continuous monitoring of chemical concentration in industrial, chemical and biochemical processes. As such, the TAS concept has many potential applications in biotechnology, process control, as well as the environmental and medical sciences.

Fabrication methods currently involve the use of photolithography to either generate a mould in which the actual chip of a polymeric material is made or to fabricate the microchip directly. Chips with such planar structures have been developed in which a number of trenches or channels are fabricated in parallel. Typically, such a planar structure is

produced by etching trenches into a semiconductor substrate, such as a Silicon wafer, and then covering the etched surface by a cover plate to complete the channels. Such structures are, however, rather expensive to produce. Furthermore, since the etched substrate is most often opaque, the material *per se* is unsuitable for direct observation of channels or for optical detection of samples. Therefore, other substrate materials like glass, polydimethylsiloxane (PDMS) and polymethylmethacrylate (PMMA) are utilised. Such chips are relatively cheap to produce, optionally permitting a disposable type of products and could provide branched flow channels and exhibit local surface characteristics.

The most widely used substrate for the substrate and microchip is glass. In order to prevent fluid leakage between adjacent conduits or conduit portions, the glass substrate is bonded to a glass cover plate. In general, bonding methods including thermally cured gluing agent (Clinical Chemistry 44:11, 2249-2255, 1998), anodic bonding (J Appl Phy 54(5), May 1983) and thermal bonding (Anal Chem, 1996,68,2044-2053) are utilised to make the contact surfaces form permanent bonds. Polymers, eg. PDMS and PMMA (US5858188), are also used as chip substrate material. Permanent bonding was achieved via oxygen plasma (Anal Chem, 1998, 70,4974-4984). Very often, cleaning of the surfaces in a cleanroom environment is a crucial step to ensure proper bonding. Microfluidic glass structure permits extremely small conduit dimensions of a few microns in dimension.

For certain applications, such chips do not have to be permanently

bonded together but may be repeatedly brought apart and placed together. However, proper adhesion has to be achieved such that fluids in the channels do not leak out of the plates when fluids are pushed into the channels. For such requirement, PDMS is often the preferred choice of material. PDMS chips are often made by using a Si or a glass mould on which the PDMS prepolymer is being casted.

Injection-moulded plastic (PMMA) chips (Clinical Chemistry 44:11 2249-2255,1998 and Anal Chem, 69,2626-2630, 1997) had been used to separate large biological molecules like RNA where electro-osmotic flow is not a separation requirement. Reduction of electroosmotic flow had been achieved with at least the inner surface of the channel being of polymeric material e.g. PMMA (US patent no. 5858188 and 5750015).

For analytical and/or separation purposes, it would be advantageous to incorporate electrodes in such chips. A current method used to insert electrodes into sample/buffer wells could be found in a recent publication by SC Jacobson *et al* (Anal Chem 1994, 66, 1107-1113). Others may include sputtering of metal onto chips and selectively removing portions of it by photolithography and metal etching to obtain metal lines that act as electrodes. These metal lines on the chips lead from wells to metal pads and can be connected to electrical power supply (Anal Chem 1997, 69, 3153-3160).

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A-E show the structures formed during the various steps of the substrate fabrication process according to one embodiment of the

present invention. Fig.1A and 1B are the perspective view and cross-sectional view along line A-A' of a negative relief pre-mould respectively. Fig. 1C and 1D are the perspective view and cross-sectional view along line A-A' of a positive relief mould respectively. Fig.1E is a substrate formed by this process.

Figure 2 is a positive relief mould formed using another method of substrate fabrication according to the present invention.

Figure 3A shows an intermediate step in the fabrication process of electrodes on the substrate.

Figures 3B and 3C are schematic drawings of substrates fabricated according to the process shown in Fig. 3A.

Figure 4 is a schematic drawing to show a chip and its internal features produced according to the present invention.

Figure 5A and 5B show the various layers laminated together to form two chips according to the present invention.

Figure 5C shows another chip produced according to the present invention.

SUMMARY OF THE INVENTION

It is an object of the present invention to provide a leak-proof microfluidic structure and method of producing the same.

Accordingly, the present invention provides, in one aspect, microfluidic structures with at least one internal channel for fluid retention. In one embodiment, the microfluidic structure is a card-like chip

comprising a substrate and a cover laminated thereon. The substrate is preferably a thin planar structure with at least one fluid channel formed on at least one side. A flexible chip is produced when a thin glass plate is used as the substrate. In another embodiment, the chip is an integral piece of plastic containing internal channels for fluid retention. This plastic chip may be provided with access holes for external access to the channels, and electrically conductive electrodes to allow electrical connection between fluid in the channel and the exterior. The plastic chip may be made of thermoplastic or thermoset resin.

In another aspect, methods are provided for producing the aforementioned substrates and chips. In one embodiment, a thermoset plastic substrate with formed features on at least one side is made using a mould with a positive relief pattern. A cover made from a similar thermoset plastic is then placed on the substrate and the two structures cured together using either a layer of uncured plastic of the similar kind or a curing agent suitable for curing both structures. In another embodiment, the substrate and the cover are made from thermoplastic, and the two pieces are bonded together under the appropriate temperature and pressure. The resulting chip forms an integral piece of plastic with internal channels formed therein.

In a third aspect, methods are provided for the production of the substrate with the desired features. In one embodiment, a plastic mould with a positive relief pattern is produced from a pre-mould containing a negative relief pattern. The pre-mould is produced by conventional methods such as photolithography. The substrate can then be formed

using the plastic mould. To produce a thermoset plastic substrate from the same thermoset plastic mould, a layer of metal is first provided on the mould, followed by additional of uncured thermoset resin to produce the substrate. To produce a thermoplastic substrate from a thermoset plastic mould, a piece of thermoplastic material is heat pressed at the appropriate temperature and pressure onto the mould to form the substrate with the desired features.

DESCRIPTION OF THE INVENTION

The following detailed description describes the preferred embodiment for implementing the underlying principles of the present invention. The microfluidic structures that are produced would become apparent from the methods described below. One skilled in the art should understand, however, that the following description is meant to be illustrative of the present invention, and should not be construed as limiting the principles discussed herein. In addition, certain terms are used throughout the following description and claims to refer to particular components. As one skilled in the art will appreciate, companies may refer to a component by different names. This document does not intend to distinguish between components that differ in name but not in function.

A chip refers to a planar structure containing microfluid channels. A chip typically comprises a substrate bonded to a cover. A substrate refers to the planar structure on which channels are formed. The cover is typically a sheet or plate that may be bonded to one or both sides of the

substrate. A negative relief mould refers to a mould with the identical features as the substrate. A positive relief mould refers to a mould with the complementary features as the substrate. Thus, for a substrate with recessed channels, a negative relief mould contains the identical recessed features, while a positive relief mould contains the complementary raised features. Thermoplastics refer to a plastic that can be repeatedly softened by heating and hardened by cooling through a temperature range that is characteristic of the plastic and that in the softened state can be shaped into an article by moulding under apropriate conditions. Thermoset plastics refer to plastics that contain reactive resins that can be cross-linked by a curing agent to form a cured rigid or flexible polymer. In the claims the terms "including", "having" and "comprising" are used in an open-ended fashion, and thus should be interpreted to mean "including but not limited to .....".

In the following drawings, only one branched channel is shown for ease of illustration. It should be understood that numerous channels of various types and shapes could be formed. The channels are generally for chemical and biological uses, such an diagnostic and purification apparatus. The channels may be of any size, but typically are above 1μm in diameter and preferably below 1mm in diameter. For microfluidic applications, the channels may also be above 1mm in diameter.

FABRICATION OF SUBSTRATE

Figures 1A to E show one method of producing a substrate according to the present invention. In this embodiment, a pre-mould **12** with negative relief pattern **10** is used. The pre-mould may be made of

materials like Si, plastic materials, e.g. PMMA or any other suitable materials. PDMS prepolymer is poured onto the pre-mould **10** and allowed to cure at a temperature of between about 25 to 80^{0}C for a duration of between about 10min to 24 hours. After curing, the cured PDMS mould **18** is peeled off from the pre-mould. PDMS mould **18** to form a will have positive relief pattern **14** complementary to the one required in the final substrate. A thin layer of metal **16** is then sputtered onto PDMS mould **18** a metal-coated PDMS mould **19** with positive relief pattern. A small amount of PDMS prepolymer is then poured onto the metal-coated PDMS mould **19**. The whole assembly is then allowed to cure at a temperature of between about 25 to 80^{0}C for a duration of between about 10min to 24 hours. After curing, the cured PDMS substrate **20** is then peeled off from the metal-coated PDMS mould **19**. Figure 1E shows PDMS substrate **22** with feature formed on one side.

Referring now to Figure 2, another embodiment of the present invention uses a PMMA positive relief mould **24** with the complementary raised feature **26** to produce a PDMS substrate. In this embodiment, uncured PDMS is poured onto PMMA mould **24** and allowed to cure after which the cured PDMS substrate with the formed channel is removed from the PMMA mould **24**. This PDMS substrate has identical features to the one shown in Figure 1E.

In a third embodiment, a flat piece of PMMA can be used as the substrate. Laser writing or other engraving or etching methods is performed on this piece of PMMA substrate to give the desired pattern, similar to the structure obtained in Figure 1E.

In yet another method, the features required are formed on a PMMA substrate by a heating and pressing step. In this embodiment, a glass plate with a positive relief pattern is used as a mould whereon a blank piece of PMMA substrate is pressed. The pressing is performed with a pressure loading of at least 10 psi and with heating at a temperature between about 150 to 220^{0}C. This treatment causes the pattern on the glass substrate to be transferred over to the PMMA substrate, giving the desired feature.

For electrophoretic purposes, the substrate is further provided with electrically conductive electrodes such as metallic strips of Au or Pt. As shown in Figure 3A, a mask **34** with openings **32** corresponding to the desired electrode patterns is placed on top of a substrate **35** with channels **37**. The mask may be made of PMMA, transparencies, plastic sheets, metal or any other suitable material. The substrate may be any polymer like PDMS or PMMA. Metal is then sputtered onto the substrate **35** with the mask **34** placed on top. The mask **34** is then removed after the sputtering to achieve a structure as shown in Figure 3B. Sputtered metals **38** serve as electrodes. The same process may be applied to the fabrication of electrodes on the covers and substrates made of glass. Figure 3C shows a cover **36** which electrodes **38** are formed.

In yet another embodiment (not shown), a layer of metal is first sputtered onto the substrate after which a layer of photoresist is spin-coated on top of the metal. The photoresist is exposed under UV light using a metal mask with a desired pattern. After exposure, the photoresist is developed and the exposed metal is etched away. The

unexposed photoresist is then removed using a suitable solvent, e.g. acetone or chloroform or suitable plasma chemistry like oxygen plasma. The metallic strips are then exposed and the final substrate formed has features similar to the ones shown in Fig.3B and 3C.

In a similar method as described above, photoresist can first be spin-coated onto the surface of a blanked substrate. If PDMS is used as the substrate material, the surface must first be plasma-treated(oxygen plasma) in order to modify the surface to become hydrophilic. The substrate with the photoresist is then exposed under the UV light or other light source of suitable wavelength through a suitable mask and then developed to remove the exposed photoresist. Metal is then sputtered onto the regions of the substrate whereby there is no photoresist and any excess photoresist is then removed using acetone or chloroform or suitable plasma chemistry like oxygen plasma. The final substrate formed is similar to the ones shown in Fig. 3B and 3C.

FABRICATION OF CHIP

To fabricate the chip, a cover has to be provided to close the channels formed on the substrate. Different methods may be used to provide a leak-proof seal of the channels, depending on the type of material used to make the substrate. In many applications, holes are first formed on the cover to act as access holes for the channels in the formed chip. The holes may be simply punched in the cover used to close the channels of the substrate.

THERMOSET PLASTIC CHIP

Referring first to Figure 4, in which PDMS is used as the material for the substrate, a thin layer of fresh and uncured PDMS is applied onto the featured surface **40** of substrate **42**. In this example, the channels **46** are provided with electrodes **48**. The uncured PDMS is spread across the surface **40** evenly by either spin-coating or simply using a ruler to brush across the surface to smoothen and remove the excess PDMS at the same time. Cover **50** containing pre-punched holes **44** is then placed on top of substrate **40** with proper alignment. Compressed gas may be blown through each of the holes **44** to further prevent any blockage of the microchannels from the curing PDMS. The whole system is then placed in the oven to cure at a temperature of between about 25 to 80^{0}C for a duration of between about 10min to 24 hours. Besides using uncured PDMS, a small fresh amount of curing agent can also be applied between substrate **40** and cover **50** to cause bonding. The resulting chip is substantially one integral piece of PDMS plastic chip of a uniform material with internal channels (and, optionally, electrodes) provided therein.

THERMOPLASTIC CHIP

If the substrate and the cover are both made of thermoplastic such as PMMA, the two parts are thermal bonded together under appropriate conditions. For example, for PMMA, placed in the oven with a pressure loading of between 5 - 10 psi to effectively induce thermal bonding at between about 150 to 220^{0}C. Alternatively, some suitable solvent can be

used to dissolve a thin layer of PMMA in either the substrate or the cover, and the two structures are then placed together. Thermal bonding will occur when the two structures are allowed to stand either at ambient temperature or at an elevated temperature of between about 150 to 220^{0}C. A chip similar to the one shown in Figure 4 is then formed. The resulting chip is again substantially one integral piece of PMMA plastic chip with internal channels (and, optionally, electrodes) provided therein.

In another variation, a photo-activateable glue is applied to the featured surface of the substrate before the cover is placed thereon. A mask containing the same features is then placed on the cover. A device providing photons in the activation wavelength is then directed at the cover and substrate such that the photo-activateable glue would be cured at the prescribed surfaces. The glue located at the features would not be cured due to the protection of the mask from the activating photons. After photoreaction, a suitable solvent is then used to remove the non-activated glue. In this embodiment, the cover or substrate should be transparent to the activation wavelength, and may be made from the same material as the glue.

LAMINATED CHIP

Referring now to Figure 5A, yet another embodiment is provided for the fabrication of a chip. In this embodiment, the substrate may be either a flexible material such as polymer, thermoplastic or thermoset resin, or of rigid material such as glass and Silica. The substrate 60 with channels 62 are placed between 2 sheets of thermoplastic **64 and 66**. The

thermoplastic sheet may be of the type commonly used for lamination of cards and paper, such as polyester and Mylar™. The top sheet 64 directly above the channel has holes **68** at appropriate positions to act as openings to the channels. Heat lamination using a conventional lamination machine of the prescribed thermoplastic results in bonding of the three layers. The substrate may be any thickness depending on the requirements of different application. Typically, the thickness is 10-200μm. When the substrate is of few a microns thick. e.g. 10-30μm, a card size chip is produced. Using this method, a flexible chip is obtained, even with a substrate is made from a rigid material such as silica or glass. The substrate in the specific example described in Fig.5A is 15μm thick.

For electrophoresis, metallic electrodes **70**, can be fabricated at the ends of channel **71** on substrate **72** before lamination, as shown in Figure 5B. In this example, holes **74** are provided on the plastic cover **76** to coincide with the electrodes, allowing electrodes **70** to connect to external circuitry. As in the previous embodiment, substrate **72** is sandwiched between plastic sheets **76** and **78** and bonded together by lamination.

In a further embodiment as shown in Figure 5C, a microprocessor **58** can be co-bonded with the chip **60** for controlling the operations of the chip. For example, specific and unique instructions may be programmed into microprocessor **58** to electronically control the entire capillary electrophoresis process within channel **62** and to provide a unique separation procedure to be carried out on the chip.

The above discussion gives specific examples of performing the present invention. It is clear, however, that many variations are possible, and different material may be fabricated under different conditions. For example, the curing of PDMS can occur at temperatures of up to 200°C, and the curing time may be any period above 1 minute. The pressing of a blank piece of PMMA onto a positive relief mould to form the desired pattern may occur at any suitable temperature, e.g. 100-200°C, and any suitable pressure, e.g. above 2psi. Polycarbonate may be used as a material for the substrate.

While the present invention has been described particularly with references to the aforementioned figures with emphasis on a capillary electrophoresis system, it should be understood that the figures are for illustration only and should not be taken as limitation on the invention. In addition it is clear that the method and apparatus of the present invention has utility in many applications where retention and manipulation of fluid is required. It is contemplated that many changes and modifications may be made by one of ordinary skill in the art without departing from the spirit and the scope of the invention described.

CLAIMS

1. A microfluidic structure comprising :

a substrate having opposing first and second sides and containing at least one channel thereon, said channel adapted for retaining a fluid;

a plastic cover laminated onto said first and second sides, said thermoplastic cover having at least one hole adjacent said channel for external access to said channel.

2. A microfluidic structure according to claim 1 wherein said substrate is made of glass, polymethylmethacrylate or silica.

3. A microfluidic structure according to claim 1 wherein said plastic is a thermoplastic.

4. A microfluidic structure according to claim 3 wherein said thermoplastic is a polyester

5. A microfluidic structure according to claim 1 wherein said substrate is further provided with at least one electrically conductive electrode, said electrode electrically connecting said channel and further accessible from the exterior of said structure, whereby electrical connection may be achieved between said channel and an external power source.

6. A microfluidic structure comprising :

an integral piece of plastic containing at least one internal channel therein, said channel for fluid retention; and at least one hole connecting said channel to the exterior.

7. A microfluidic structure according to claim 6 further comprising at least one electrically conductive electrode disposed on said channel, said electrode accessible from the exterior of said structure whereby electrical connection may be made between said channel and an external power source.

8. A microfluidic structure according to claim 6 or 7 wherein said plastic is a thermoset plastic.

9. A microfluidic structure according to claim 6 or 7 wherein said plastic is a thermoplastic.

10. A microfluidic structure according to claim 6 or 7 wherein said plastic is polydimethylsiloxane or polycarbonate.

11. A microfluidic structure according to claim 6 or 7 wherein said plastic is polymethylmethacrylate.

12. A method of producing a microfluidic structure comprising :

providing a plastic substrate with features on at least one surface;

aligning a plastic cover on said surface with features, said plastic cover made of the same material as said substrate;

bonding said cover onto said substrate to produce an integral piece of plastic chip with internal spaces adapted for fluid retention.

13. A method according to claim 12 wherein said plastic cover comprises at least one hole, said feature comprises at least one channel, said hole adjacent said channel to provide external access into said channel.

14. A method according to claim 12 wherein said plastic cover comprises at least one hole, said feature comprises at least one channel, said hole adjacent said channel to provide external access into said channel; said method further comprising the step of providing electrically conductive electrodes for electrical connection between said fluid and the exterior of said chip.

15. A method according to claim 13 wherein said plastic is a thermoset plastic, and said bonding method comprises administering a thin layer of the same thermoset plastic in an uncured form between said cover and said substrate, and allowing curing to occur without blocking said channel.

16. A method according to claim 13 wherein said plastic is a thermoset plastic, and said bonding method comprises administering a thin layer of the curing agent for the same thermoset plastic between said cover and said substrate, and allowing curing to occur without blocking said channel.

17. A method according to claim 13 wherein said plastic is polydimethylsiloxane, and said bonding method comprises administering a thin layer of the curing agent for the same thermoset plastic between said cover and said substrate, and allowing curing to occur without blocking said channel.

18. A method according to claim 13 wherein said plastic is a thermoplastic, and said bonding method comprises thermal bonding

at a temperature and pressure prescribed for said thermoplastic without blocking said channel.

19. A method according to claim 13 wherein said plastic is polymethylmethacrylate, said feature is a channel, and said bonding method comprises thermal bonding at a temperature and pressure prescribed for said thermoplastic without blocking said channel.

20. A method according to claim 13 wherein said bonding step comprises

administering a thin layer of a photoactivateable plastic in an uncured form between said cover and said substrate;

protecting said uncured plastic with a mask containing said features;

producing a source fo photons with a wavelength capable of activating and curing said photoactivateable plastic; and

curing said photoactivateable plastic at the unprotected sites; and

removal uncured photoactivateable plastic.

21. A method according to claim 20 wherein said plastic is the same photo-activateable plastic.

22. A method of producing a microfluidic structure comprising :

providing a planar substrate having opposing first and second surfaces, said substrate having features on at least said first surface;

laminating said first and second surfaces with thermoplastic.

23. A method according to claim 22 wherein said substrate is made of glass, and channels are etched onto said glass.

24. A method of producing a microfluid substrate comprising :

providing a pre-mould with a negative relief pattern;

making a thermoset plastic mould from said pre-mould;

providing a layer of metal onto said plastic mould;

forming a thermoset plastic substrate with the prescribed pattern.

25. A method of producing a microfluid substrate comprising :

providing a pre-mould with a negative relief pattern;

making a thermoset plastic mould from said pre-mould;

pressing a piece of thermoplastic onto said mould under predetermined temperature and pressure to form a substrate with the prescribed pattern.

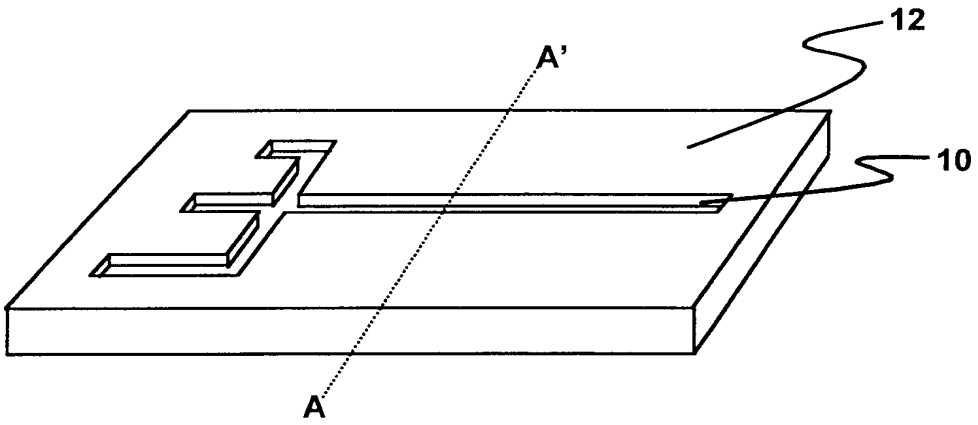

FIG. 1A

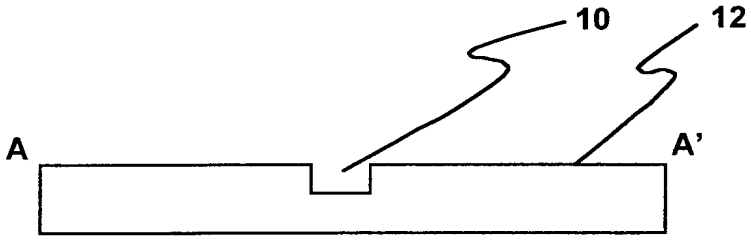

FIG. 1B

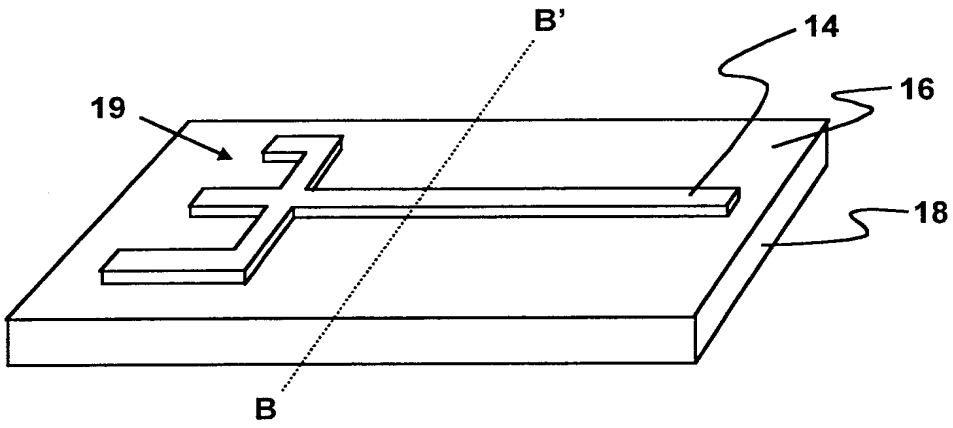

FIG. 1C

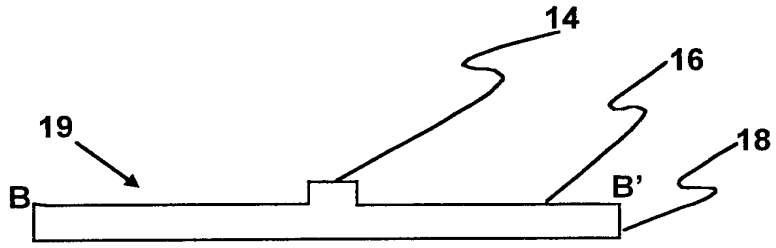

FIG. 1D

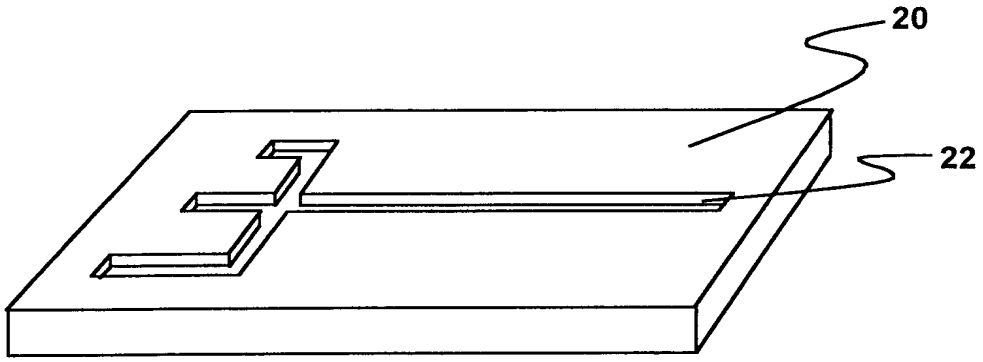

FIG. 1E

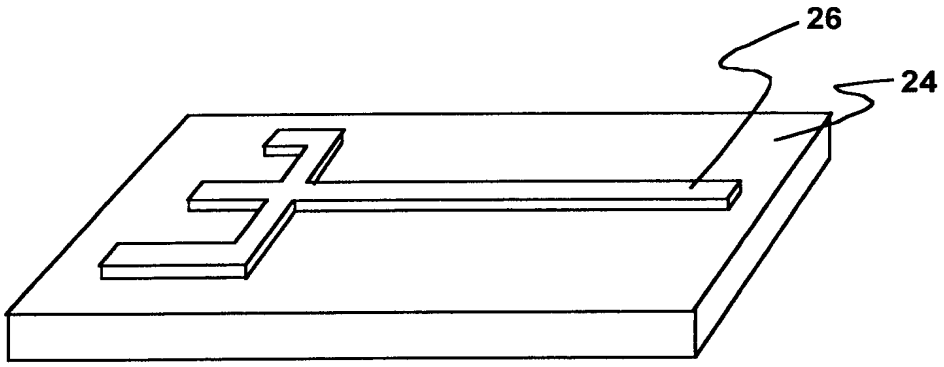

FIG. 2

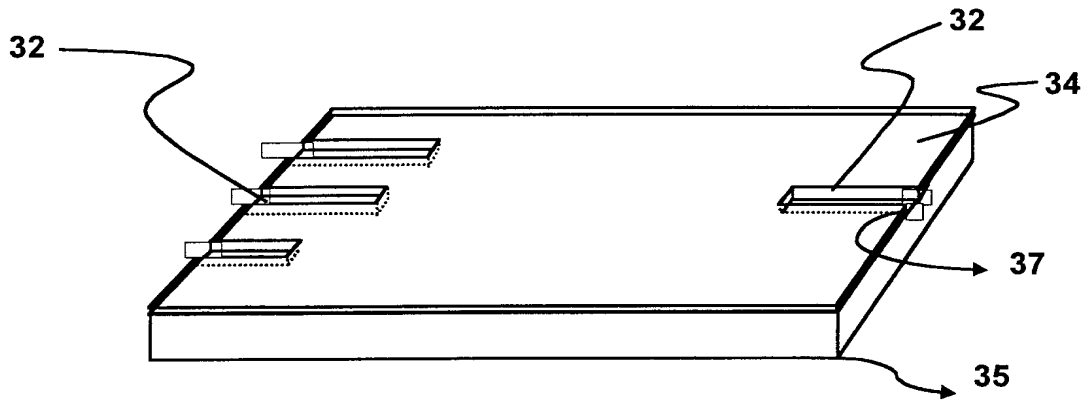

FIG. 3A

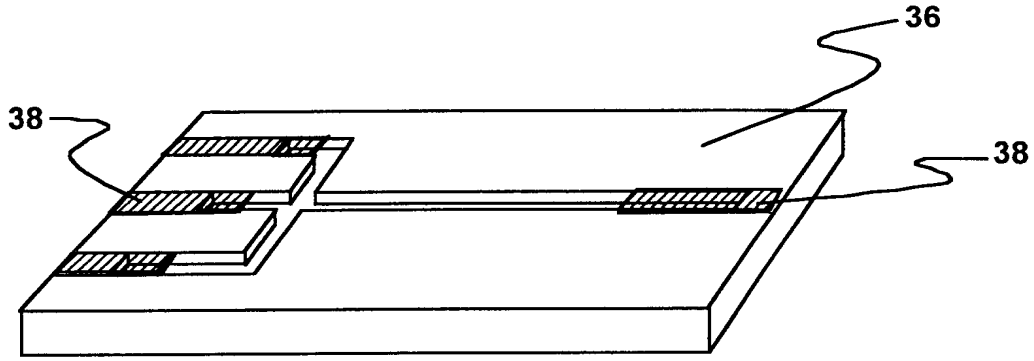

FIG. 3B

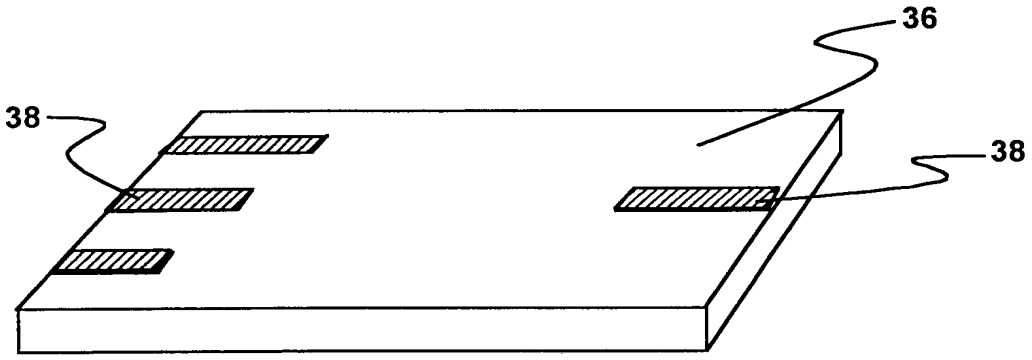

FIG. 3C

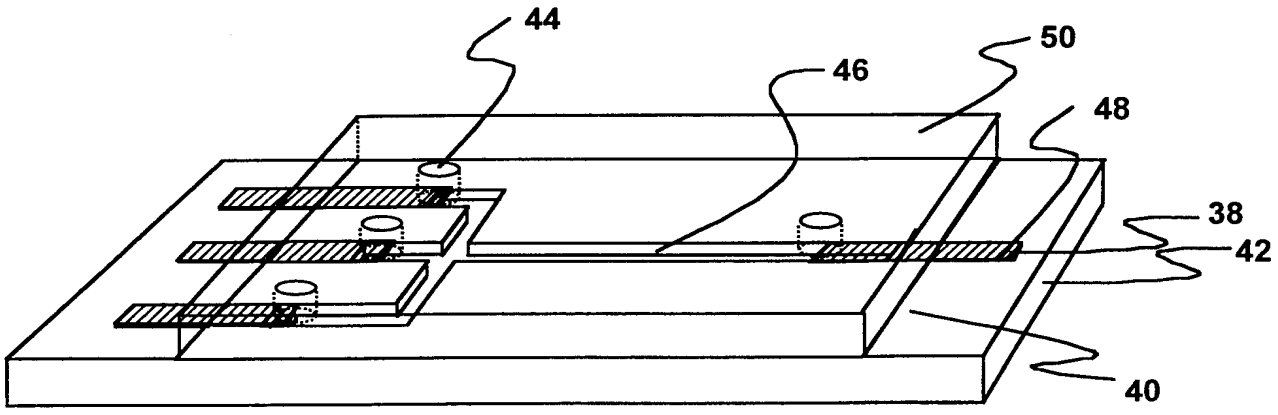

FIG. 4

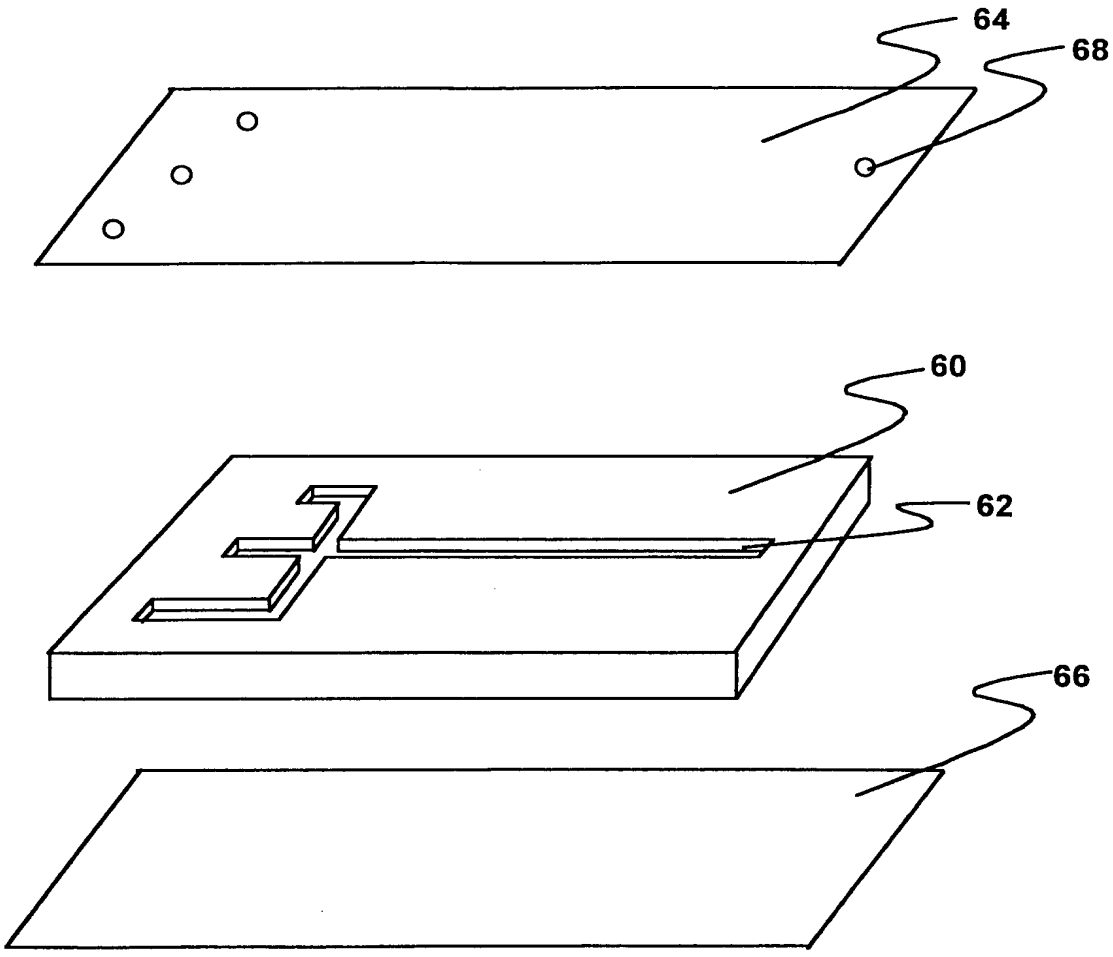

FIG 5A

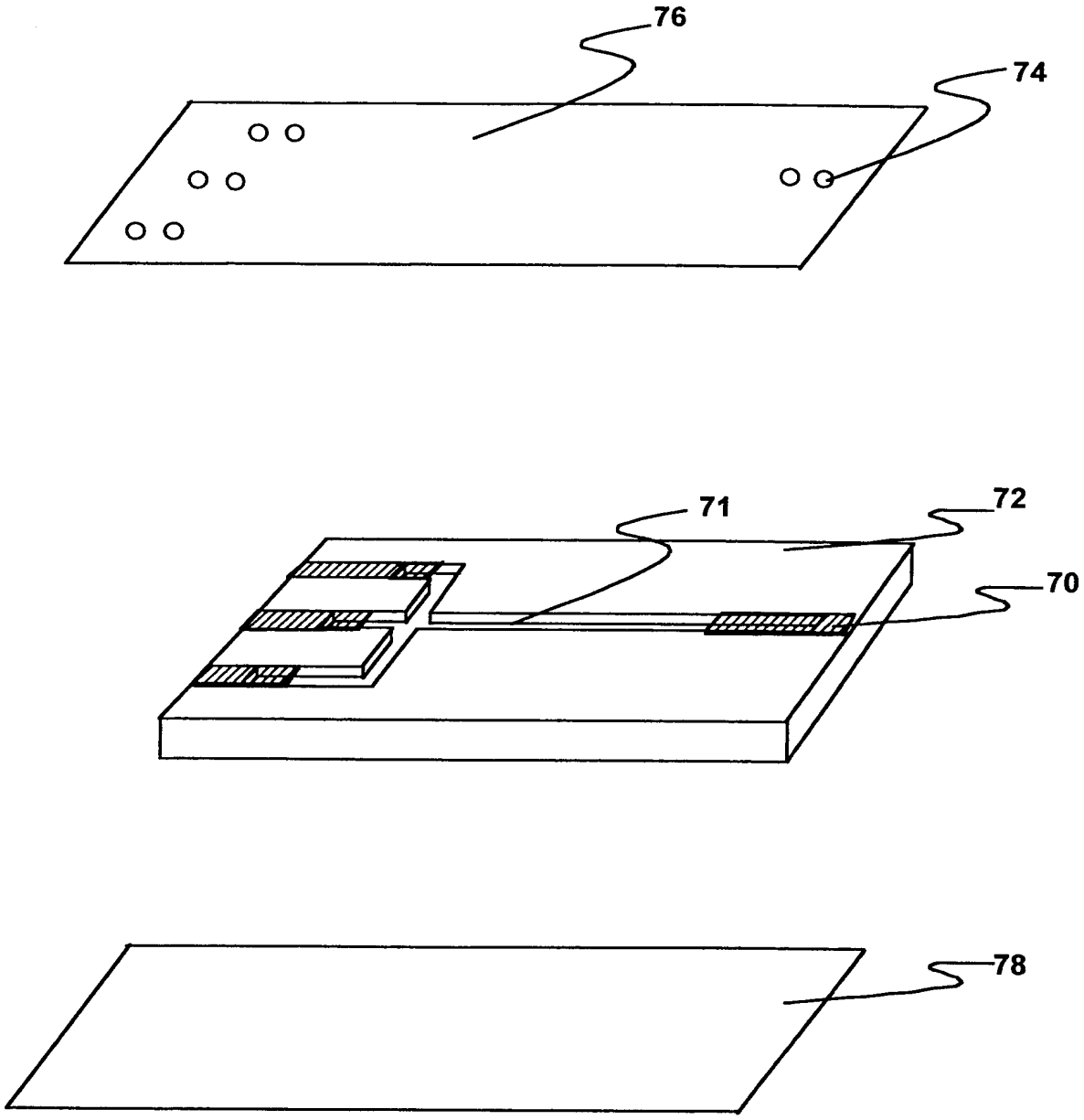

FIG 5B

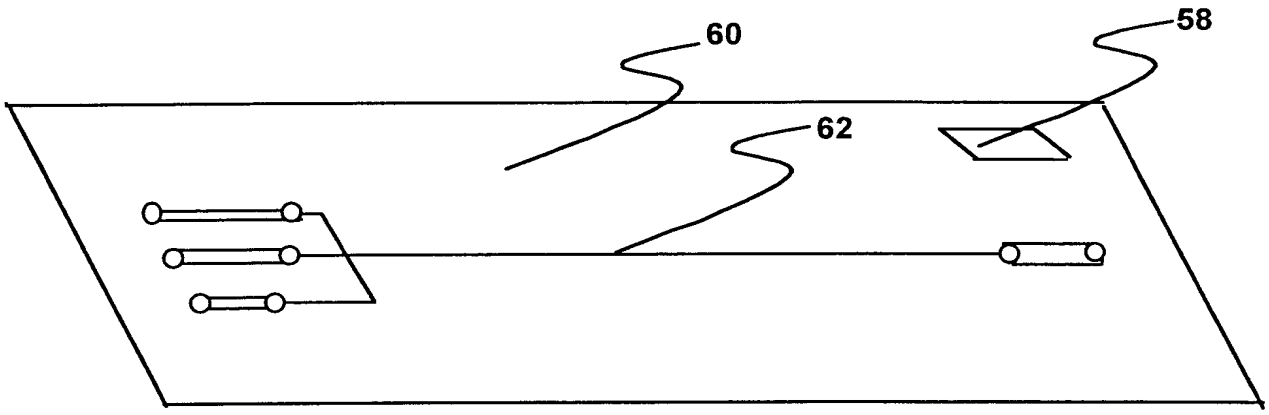

FIG 5C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SG 00/00159

CLASSIFICATION OF SUBJECT MATTER

IPC[7]: B01L 3/00 G01N 27/447 B32B 3/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC[7]: B01L 3/00 G01N 27/447 B32B 3/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DE 198 16 224 A1(OLYMPUS) 15 October 1998 (15.10.98) abstract,fig. 6	1,6,12,22,24, 25
A	WO 98/39645 A1(BECKMAN INSTRUMENTS) 11 September 1998 (11.09.98) abstract,figs	1,6,12,22,24, 25
A	WO 91/16966 A1(PHARMACIA BIOSENSOR) 14 November 1991 (14.11.91) abstract,fig. 4	1,6,12,22,24, 25
A	WO 99/29497 A1 (CALIPER) 17 June 1999 (17.06.99) abstract,fig. 5	1,6,12,22,24, 25

☐ Further documents are listed in the continuation of Box C. ☒ See patent family annex.

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„&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 January 2001 (26.01.2001)	26 February 2001 (26.02.2001)
Name and mailing adress of the ISA/AT Austrian Patent Office Kohlmarkt 8-10; A-1014 Vienna Facsimile No. 1/53424/535	Authorized officer NARDAI Telephone No. 1/53424/347

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/SG 00/00159

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